

REMARKS

In the Office Action mailed July 28, 2003 for the above-identified application, the restriction requirement has been made final. Claim 16, drawn to a non-elected invention, has been cancelled without prejudice. Applicant reserves the right to file a divisional application directed to the subject matter of claim 16.

The Examiner has requested correction of the benefit statement to include the filing date of the provisional application. The first paragraph of the specification has been amended accordingly.

The Examiner has alleged that the present application contains sequence disclosures but fails to comply with the requirements of 37 CFR §§ 1.821-1.825. Submitted herewith is a Sequence Listing in paper and computer readable formats. The undersigned states that the content of the paper and computer readable formats is the same and contains no new matter. The application has been amended to include the Sequence Listing and sequence identification numbers.

The abstract of the invention has been amended in view of the Examiner's suggestion.

Claim 13 has been objected to as allegedly informal. The Examiner has suggested that the word "be" could be inserted between "will not" and "preferentially amplified" in line 3. Applicants point out that this amendment of Claim 13 was made by the Preliminary Amendment filed April 11, 2002. Withdrawal of the objection to Claim 13 is respectfully requested.

Claims 1-15 and 17-23 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. The Examiner has alleged that Claims 1-15 are indefinite because it is unclear whether the claims are directed to methods of determining the frequency of an allele in a population, or methods of obtaining a pattern of nucleotide incorporation. Claim 1 has been amended to recite a step of "determining the frequency of the allele from the pattern of nucleotide incorporation." Support for the amendment of Claim 1 may be found in the specification, for example, at page 6, lines 9-10.

Claims 2-6 and 18-23 are allegedly indefinite for failing to provide proper antecedent basis for the term “reaction mixture.” Claims 1 and 17 have been amended to provide antecedent basis for “reaction mixture.”

Claims 11-15 are allegedly indefinite for failing to provide antecedent basis for the term “said calibration” and as unclear in the terms “(or marker)” and “said primer extension reaction.” Claim 11 has been amended to delete the terms “said calibration” and “(or marker)” and to clarify that the primer extension reaction is the reaction used to determine the concentration of nucleic acid in the sample.

Claims 14 and 15 are allegedly indefinite as to how these claims relate back to Claims 1 and 11. The Examiner has alleged that it is unclear whether Claim 14 further defines the method of Claim 11, or whether the steps in Claim 14 are additional steps. Further, the Examiner has alleged that Claim 15 is unclear in the requirement that the sample molecules are pooled individually with the reference samples, since in Claim 1 pooling of a population is required.

Claim 14 further defines the method of Claim 11. Claim 14 has been amended to clarify that the reference sample contains the reference polymorphism. Applicants submit that it is clear from Claim 15 that the pooling of the sample nucleic acid with the reference sample refers to the calibration step of Claim 8. From the language of Claim 8 it is clear that the pooling of sample nucleic acid and reference sample occurs in the calibration step of Claim 8 that is “prior to pooling” performed in Claim 1.

Claims 17-23 are allegedly indefinite for failing to recite essential steps. The Examiner has alleged that it is unclear how the amount of occurrence of the allele is determined based upon the determination of which nucleotide is incorporated. Applicants respectfully submit that Claim 17 recites the essential steps necessary to practice the invention. The amount of allele is determined from the step of analyzing the nucleotide incorporation information. This step is recited in amended Claim 17 after the step of determining the type and number of nucleotides incorporated. The specification describes the step of analyzing the information, for example, at page 54 of the substitute specification. Accordingly, Claim 17 recites all the essential steps necessary to practice the claimed method. Further, when read in view of the teachings of the specification, Claim 17 is clear and definite to one of ordinary skill in the art.

The Examiner has further alleged that the term “said reaction” in Claim 17 is unclear. Claim 17 has been amended to replace the term “said reaction” with the term “said primer extension products.” The term “said nucleotide incorporation information” allegedly lacks antecedent basis. Claim 17 has been amended to replace this term by “the type and number of nucleotides incorporated.” Further, Claim 17 has been amended to clarify that in the last recited step, the amount of the allele is determined by analyzing the type and number of nucleotides incorporated at the polymorphic position of interest.

In view of the foregoing comments and amendments, it is submitted that Claims 1-15 and 17-23 are clear and definite to one of ordinary skill in the art. Withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Claims 1-3, 7 and 17-19 have been rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 6,287,778 to Huang et al. (“Huang et al.”). The Examiner has alleged that Huang et al. teach a method of determining the genotype of one or more individuals at a polymorphic locus comprising performing allele-specific PCR, and detecting a primer extension product, thereby obtaining a pattern of nucleotide incorporation.

Applicant respectfully submits that Huang et al. fail to anticipate the subject matter of the present invention. Huang et al. disclose a method for determining the genotype of an individual at a polymorphic locus using amplification with allele-specific extension primers. As shown in Fig. 1 of Huang et al., the extension primer terminates on the polymorphic locus, and primer extension will only occur if there is a base match between the 3' nucleotide of the primer and the polymorphic locus. Thus in the method of Huang et al., the frequency of an allele is determined based upon whether any extension occurs.

In contrast, in the method of the present invention, the primer binds adjacent to but not over the polymorphic locus. Added nucleotides are incorporated only if they are complementary to the template. As the primer extension reaction is performed, the primer is extended over the polymorphic position. Thus in the present invention, the frequency of an allele is determined based upon the pattern of nucleotide incorporation at the polymorphic locus.

In the interest of advancing prosecution, Claims 1 and 17 have been amended to recite that the primer binds at a site “substantially adjacent to a polymorphic position of interest.”

Support for the amendment of Claims 1 and 17 may be founding the specification, for example at pages 23 and 27 of the substitute specification. Claims 1 and 17 have been further amended to clarify that the frequency of the allele is determined from the pattern of nucleotide incorporation at the position corresponding to the polymorphic position of interest. Support for this amendment may be found in the specification, for example at page 30 of the substitute specification. Huang et al. neither teach nor suggest a method in which the primer binds adjacent to, but not over, the polymorphic locus, or a method in which the frequency of an allele is determined by analyzing nucleotide incorporation.

In view of the foregoing remarks and amendments, withdrawal of the rejection of Claims 1-3, 7 and 17-19 under 35 U.S.C. § 102(e) in view of Huang et al. is respectfully requested.

Claims 1-5, 7 and 17-21 have been rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Nyren et al. (1997) Analytical Biochemistry 244:367-373 ("Nyren et al."). The Examiner has alleged that Nyren et al. teach a method of detecting a single-base change using an enzymatic luminometric inorganic pyrophosphate detection assay.

Applicants respectfully submit that Nyren et al. fail to anticipate the present invention. Like Huang et al., the method of Nyren et al. utilizes primers having 3' termini over the base of interest in the template. Single base changes are determined based upon the extension rate of the primer. In contrast, in the present invention the primer binds adjacent to but not over the polymorphic position of interest. Single base changes, i.e., allele frequencies, are determined by the pattern of incorporation of added nucleotides in accordance with the present invention. Accordingly, withdrawal of the rejection of Claims 1-5, 7 and 17-21 under 35 U.S.C. § 102(b) in view of Nyren et al. is respectfully requested.

Claims 1-3, 7, 8 and 17-19 have been rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Shaw et al. (1998) Genome Research 8:111-123 ("Shaw et al."). The Examiner has alleged that Shaw et al. teach a method of determining the frequency of alleles in a pooled DNA sample by pooling nucleic acid molecules, performing primer extension reactions using primers which hybridize to the nucleic acid and obtaining a pattern of nucleotide incorporation.

Applicant respectfully submits that Shaw et al. fail to anticipate the method of the present invention. In the method of Shaw et al., PCR primers are used to amplify a region of DNA

containing a microsatellite. One of the PCR primers is fluorescently labeled. The microsatellites are characterized by different numbers of repeat motifs (n), which in turn correspond to different alleles, as shown in Table 1 of Shaw et al. Thus after amplification by PCR, the fragments are of different lengths, depending upon the allele present. The length of the fragment is determined by gel electrophoresis, and thus “n” is calculated. The size of the amplified fragments, and not sequence content, is used to determine allele frequency.

In contrast, in the method of the present invention, sequence information is obtained after nucleotide addition and is used to determine allele frequency. The primer extension reaction is performed by sequentially adding nucleotides and determining the incorporation or non-incorporation of each nucleotide. This step of “sequencing-by-synthesis” is disclosed in the specification, for example at page 6, paragraph 11 of the substitute specification. Claims 1 and 17 have been amended to clarify this step. Claims 2 and 18 have been cancelled without prejudice.

Shaw et al. neither teach nor suggest a method of determining the frequency of an allele in which incorporation or non-incorporation of sequentially added nucleotides is determined as each nucleotide is added. Withdrawal of the rejection of Claims 1-3, 7, 8 and 17-19 under 35 U.S.C. § 102(b) in view of Shaw et al. is respectfully requested.

Claims 1-3, 8, 9 and 17-19 have been rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Germer et al. (2000) Genome Research 10:258-266 (“Germer et al.”). The Examiner has alleged that Germer et al. teach a method for determining the allele frequency of biallelic populations in pooled samples.

Germer et al. disclose a method of determining allele frequency by PCR using an allele-specific primer. As disclosed by Germer et al. at page 259, the primer has a 3' end that is “directly over and matching one or the other of the variant nucleotides.” By using allele-specific primers, only completely matched primers are extended efficiently enough to create detectable amplification product. PCR amplification products are detected using fluorescent dsDNA binding dyes. The number of PCR cycles required to reach a detectable amount of fluorescence is used to calculate allele frequency.

In contrast, in the method of the present invention, the primer binds adjacent to, but not over, the polymorphic position. Further, incorporation or non-incorporation of sequentially-added nucleotides is determined as each nucleotide is added. Germer et al. neither teach nor suggest using such a primer, or determining incorporation of individual nucleotides. Withdrawal of the rejection of Claims 1-3, 8, 9 and 17-19 under 35 U.S.C. § 102(b) in view of Germer et al. is respectfully requested.

Claims 1-6, 8 and 17-22 have been rejected under 35 U.S.C. § 102(a) as allegedly anticipated by Bellman et al. (April 2000) Poster presented at Human Genome Project Meeting ("Bellman et al."). Submitted herewith is the Declaration under 37 C.F.R. § 1.132 of inventor Anna Sylvan containing a showing that: Anna Sylvan is the sole inventor of the presently claimed invention; the subject matter of Bellman et al. is the applicant's own invention; and the Bellman et al. reference discloses subject matter derived from the inventor, and not invented by the named authors of the reference. Accordingly, withdrawal of the rejection under 35 U.S.C. § 102(a) in view of Bellman et al. is respectfully requested.

Claims 1-3, 8 and 17-19 have been rejected under 35 U.S.C. § 102(a) as allegedly anticipated by Breen et al. (March 2000) BioTechniques 28:464-470 ("Breen et al."). The Examiner has alleged that Breen et al. teach a method for determining the frequency of an allele in a population of nucleic acids by pooling nucleic acids, performing a primer extension reaction and obtaining a pattern of nucleotide incorporation. The method of Breen et al. comprises a primer extension reaction utilizing allele-specific primers, i.e. primers that bind to the polymorphic position of interest. Further, the amount of amplification was quantitated by densitometric analysis or allele-specific fluorescent probes in order to calculate allele frequencies. In contrast, the present method utilizes primers that bind adjacent to the polymorphic position, and utilizes nucleotide incorporation information to calculate allele frequencies. These features are neither taught nor suggested by Breen et al. Accordingly, withdrawal of the rejection under 35 U.S.C. § 102(a) in view of Breen et al. is respectfully requested.

Claims 6, 22 and 23 have been rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Nyren et al. in view of WO98/28440 to Nyren ("Nyren-2"). The Examiner has alleged that Nyren et al. teach a method of detecting a single-base change using an enzymatic

luminometric inorganic pyrophosphate detection assay, and that Nyren-2 teaches the use of a nucleotide degrading enzyme in the polymerase reaction step of a method of identifying a base as a target position in a sample DNA sequence. It would have been obvious, the Examiner has alleged, to modify the primer extension assay of Nyren et al. to include a nucleotide degrading enzyme during the primer extension reaction.

Applicant respectfully submits that the combination of cited references fails to render the present invention obvious. As discussed hereinabove, Nyren et al. fail to teach or suggest a method utilizing a primer that binds adjacent to a polymorphic position of interest. Nyren et al. further fails to teach or suggest a method in which allele frequency is determined by assessing the incorporation or non-incorporation of nucleotides at a position correspond to the polymorphic position. Accordingly, even if one were motivated to modify the method of Nyren et al. with the nucleotide-degrading enzyme of Nyren-2, the present invention would not be achieved. Withdrawal of the rejection of Claims 6, 22 and 23 under 35 U.S.C. § 103(a) is respectfully requested.

Claims 9-11, 14 and 15 have been rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Breen et al. As discussed hereinabove, Breen et al. fail to teach or suggest a method in which primers bind adjacent to the polymorphic position, or a method in which nucleotide incorporation is determined to calculate allele frequencies. Accordingly, even if one had been motivated to modify the method of Breen et al. by determining the concentration of nucleic acid in the sample, one would not have achieved the present invention. Withdrawal of the rejection under 35 U.S.C. § 103(a) in view of Breen et al. is respectfully requested.

Claims 10-15 have been rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious by Germer et al. The Examiner has alleged that Germer et al. teach a method for determining the allele frequency of biallelic polymorphisms in pooled samples, but do not teach specifically adjusting the amount of nucleic acids. However, the Examiner has alleged that Germer et al. teach the importance of accurate quantitation of individual DNAs, and thus it would have been obvious to adjust nucleic acid concentrations.

As discussed hereinabove, Germer et al. fail to teach or suggest a method using a primer that binds adjacent to a polymorphic position of interest, or a method in which incorporation of

sequentially added nucleotides is determined as each nucleotide is added. Accordingly, even if one had been motivated to modify the method of Germer et al. by adjusting the amount of nucleic acid in the sample, one would not have achieved the present invention. Withdrawal of the rejection of Claims 10-15 under 35 U.S.C. § 103(a) is respectfully requested.

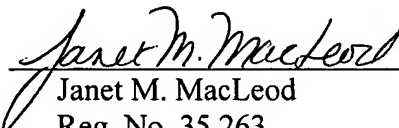
In view of the foregoing comments and amendments, it is respectfully submitted that the present application is in condition for allowance.

Favorable consideration and allowance of all pending claims is earnestly solicited.

Respectfully submitted,

DORSEY & WHITNEY LLP

Date: January 22, 2004


Janet M. MacLeod
Reg. No. 35,263
250 Park Avenue
New York, NY 10177
(212) 415-9200